Assessment of Mercury Contamination in Bats at the Great Dismal Swamp National Wildlife Refuge

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KEYWORDS: Mercury, Hg, Methylmercury, MeHg, Bats, Virginia, Great Dismal Swamp National Wildlife Refuge

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Introduction

Mercury (Hg) deposition has the potential to have landscape level impacts to mammals and birds. Atmospheric deposition of Hg is the suspected cause of Hg contamination in the Great Dismal Swamp National Wildlife Refuge (GDSNWR). Hg deposition occurs predominantly in the inorganic form (Hg), while Hg bioaccumulation is most significant in the methylated form (MeHg) (Driscoll et al. 2007). The availability of Hg is strongly influenced by hydrology and biogeochemical factors (Lucotte et al. 1999; Watras and Huckabee 1994). Due to Hg methylation by sulfate-reducing anaerobic bacteria that convert inorganic Hg into toxic organic methylmercury (MeHg), Hg is more bioavailable to wildlife in areas with low dissolved oxygen and low pH than other habitat types. Due to the low dissolved oxygen and low pH found in the wetland environment at the GDSNWR, the risk to mammals and birds from bioaccumulation of MeHg is higher than in other ecosystems.

Most studies looking at the bioaccumulation of Hg focus on the piscivorous food chain. Few studies have evaluated Hg bioaccumulation in the insectivorous food chain. Many bat species regularly forage on emerging insects over river surface waters and floodplain edges. Bats that forage over waterways with elevated Hg concentrations have been shown to bioaccumulate Hg (Yates et al. 2007; Divoll et al. 2009). Bats also have a high metabolic rate and high ingestion rate relative to their body weight, which may make them more vulnerable to bioaccumulation. Due to the high potential for bioaccumulation at the GDSNWR, measuring Hg concentrations in bats is important for understanding bioaccumulation in the terrestrial food chain and evaluating potential affects.

Tissues commonly analyzed in mammal bioaccumulation studies include blood and fur. Blood and fur represent different temporal uptake exposure routes. Blood Hg levels represent more recent dietary uptake (Evers et al. 2005; Hobson and Clark 1993, 1994; Bearhop et al. 2000) Fur samples are indicators of Hg body burdens, reflecting both dietary uptake and body accumulation since Hg is deposited in the fur as it grows over time (Mierle et al. 2000; Yates et al. 2005). Differences in blood and fur Hg concentrations through time can be a result of shifts in bat movement or prey choice.

The objective of this study was to develop a Hg exposure profile for the GDSNWR bat populations based on fur and blood samples obtained from mist netting. Because it is well-

established that Hg concentrations in fur and blood are primarily in the methyl form, these tissues are good indicators of Hg availability to bats.

Methods

Study Area

The GDSNWR is located on the Virginia-North Carolina border in the southern Atlantic Coastal Plain. The Union Camp Corporation originally donated 49,100 acres of forested wetlands to The Nature Conservancy in 1974, which was then conveyed to the Department of the Interior to establish the refuge. GDSNWR currently consists of 111,201 acres, with Lake Drummond, a 3,100 acre natural lake, in the center of the swamp. Lake Drummond acts as a sump for water flows from the north and west of the refuge. The study area encompasses sampling locations within the cities of Suffolk and Chesapeake, Virginia (Figure 1). Ditches and roads were built throughout the GDSNWR for logging purposes before the refuge was established. A combination of standing water and decomposing organic matter causes low dissolved oxygen levels and low pH throughout the waters of the GDSNWR . Surface water in the swamp is acidic, ranging from pH 3.0 to 6.0.

Bat Capture and Sampling

Bat capture and sampling occurred at eight sites during 2007, 2008, and 2009. The sample sites were Myrtle Ditch, Martha Washington Ditch, West Ditch, Washington Ditch, Jericho-Fire Tower, East Ditch, East Ditch-Railroad (RR), and East Ditch-South of RR. Six, nine and twelve meter, 36 millimeter (mm) mesh Avinet (Dryden, New York) bat-specific mist nets were hung on a 30 foot Triple High Forest Filter system (Bat Conservation and Management, Carlisle, Pennsylvania) on roads that parallel ditches in areas with suitable roosting habitat. The nets were elevated on the triple high's pulley system to block a travel corridor and capture bats in flight.

Nets were set the day before capture, opened before dusk, monitored by flashlight every ten minutes for captures, and closed when capture efforts ceased. Nets were disinfected following U.S. Fish and Wildlife Service protocols to prevent possible spread of disease between bats. All bats captured were identified to species, checked for reproductive status, sexed, banded, and aged. Age was determined by the degree of ossification in phalangeal epiphyses (Kunz 1982). In 2009, Rafinesque's big-eared bats (*Corynorhinus rafinesquii*), a state listed endangered bat were released

without bands, measurements or samples taken due to their fragile nature. Fur samples were collected with freshly cleaned, stainless steel scissors from the back and stomach areas. Blood was collected in heparinized capillary tubes from the uropatagium or wing. Capillary tubes were sealed on both ends with Critocaps® and placed in pre-labeled 10 cc plastic vacutainers. All blood, fur, and wing punch samples were stored on ice in a cooler and frozen within 6-8 hours of collection. Bats were immediately released at the capture site after all data was recorded. Samples were shipped to Texas A&M Trace Element Research Laboratory, College Station, Texas in 2007 and 2009 and Laboratory and Environmental Testing Inc., Columbia, Missouri in 2008 for total Hg analysis.

Mercury Analysis at Laboratory and Environmental Testing, Inc.

Blood and fur samples were freeze-dried and then homogenized for sample preparation.

Samples were placed in a whirl-pak®, weighed, sealed, and placed in a freezer until frozen solid.

After the samples were frozen, they were placed in the freeze-drier where the temperature reached -50°C. After samples were dried, they were removed from the chamber, weighed and percent moisture was calculated.

For microwave digestion, 0.5 grams (g) of dry sample was weighed into a clean Teflon digestion vessel and 5.0 milliliters (ml) concentrated trace metal grade nitric acid was added. After a few minutes, 1.0 ml of high purity hydrogen peroxide was added and the sample was placed in the microwave. After the microwave heating was completed and the samples cooled to room temperature, the sample was diluted to 50.0 ml with distilled water and transferred to a clean 2 ounce plastic bottle. Vessels that vented during the digestion were re-digested with either less sample or a longer ramp at lower temperatures.

For Hg analysis, a 10 ml aliquot was removed immediately after dilution and placed in a plastic tube with 100 microliters (µl) of concentrated trace metal grade hydrochloric acid. Samples were analyzed for Hg by cold vapor atomic absorption. Calibration standards were prepared with 10 percent hydrochloric acid and 5 percent stannous chloride-10 percent hydrochloric acid. Pumps were started and the tubes were placed in the hydrochloric acid and stannous chloride. A 10 or 20 parts per billion (ppb) standard was run until the sensitivity stabilized and consecutive readings varied by less than 2 percent. Calibration was done with 0, 1.0, 5.0, and 30.0 ppb standards. Quality Control checks were 10.0, 20.0, and a known reference sample. The 5.00 ppb standard was checked every 10 tubes and if it is more than 5 percent from 5.00 the instrument was recalibrated.

If the value was more than 10 percent from 5.00, then the last 10 samples were rerun. Detection limits were 0.05 ppm at Laboratory and Environmental testing. All samples from 2008 that had the minimum detection limit of 0.05 parts per million (ppm) fresh weight (fw) were divided by two to help correct for errors caused by low detection limits.

Mercury Analysis at Trace Element Research Laboratory

Hg was analyzed by cold vapor atomic absorption using a Cetac 7500 QuickTrace® analyzer equipped with a heated absorption cell and thermostatically stabilized detector block. The instrument is mated to a Cetac ASX-510® autosampler and controlled by a desktop computer using Cetac's QuickTrace software. Divalent mercury (Hg⁺⁺) in aqueous samples (water, tissue, or sediment digests) was reduced to the elemental state (Hg^o) by stannous chloride, a strong reducing agent. The fraction of Hgo that enters the gas phase was introduced into an atomic absorption cell, where light produced by a separate Hg vapor lamp was absorbed by the free Hg atoms. The amount of Hg in the sample was determined by comparing light absorption of the sample with that of calibration standards. Reagent water contained no analytes above the method detection limit. Reagent water was produced by passing deionized water through a series of polishing deionizer cartridges. Hydrochloric acid was Baker® reagent grade or equivalent and stored in the original glass bottle. The calibration solution was made from a commercially available reference standard and 7 percent hydrochloric acid. The commercial stock solution, usually obtained at a 1000 ppm concentration level, was diluted to 10 ppm and 100 ppb working standards, and subsequently diluted to lower concentration levels for calibrating the instrument. The matrix recovery spiking solution working Hg standards were typically used for spiking samples. The 10 ppm standard was generally used for tissue samples and the 100 ppb standard was used for sediment samples. These were modified as appropriate to accommodate sample concentrations and masses. The calibration check standard was NIST SRM 1641d (Hg in water) and was diluted by a factor of 500 with 7 percent hydrochloric acid and was used to verify the calibration curve. A 10 percent divalent cation tin (Sn⁺⁺) solution was used to reduce Hg⁺⁺ to Hg⁰. It was made by adding 100 g stannous chloride to 70 ml of concentrated hydrochloric acid and diluted to 1 liter. Any Hg contamination was removed by stirring the solution overnight, allowing Hg^o to escape to the atmosphere.

Statistical Analysis

All data was analyzed using JMP 9.0 Statistical Program. To improve normality of data distribution, blood and fur data was log transformed. A t-test was used to determine significant differences by age, sex, age by sex, or sex by age (p<0.05). Site and species were analyzed using a one way ANOVA. Due to their close proximity and the fact that they were both located on the same ditch, samples from East Ditch-RR and East Ditch-south of RR were pooled and named East Ditch-RR. Tukey HSD was used on species to determine which species had significant differences (p<0.05).

Results

A total of 209 bats representing seven species were captured from eight locations in the GDSNWR. Evening bats (*Nycticeius humeralis*), big brown (*Eptesicus fuscus*), and tri-colored bats (*Perimyotis subflavus*) were the most abundant species captured. No juvenile Rafinesque's big-eared bats were captured. There were differences in the total number of blood and fur samples due the release of bats that showed signs of stress. Fur was taken from 188 bats consisting of 94 adults and 94 juveniles. In adults, fur Hg concentrations ranged from a low of 1.9 ppm (fw) in a red bat (*Lasiurus borealis*) captured from East Ditch RR to a high of 49.2 ppm (fw) in a big brown bat captured at East Ditch. In juveniles, fur Hg concentrations ranged from a low of 1.09 ppm (fw) in a red bat captured from East Ditch RR to a high of 29 ppm (fw) in a big brown bat captured from Martha Washington Ditch.

Blood was taken from 177 bats consisting of 90 adults and 87 juveniles. In adults, blood Hg concentration ranged from a low of 0.18 ppm (ww) in a big brown bat captured from east ditch to a high of 0.3 ppm (ww) in a tri-colored bat captured at Jericho Ditch. Juvenile blood Hg ranged from a low of 0.03 ppm (ww) in a big brown bat captured at Martha Washington Ditch to a high of 0.20 ppm (ww) in a evening bat captured at Jericho Ditch.

There was a significant difference in fur Hg concentrations between juveniles and adults (t=-11.20, df=153.25, p=0.000), with adults having significantly higher fur Hg concentrations than juveniles (Table 1). There was no significant difference in fur Hg concentration by sex (p>0.05). Fur Hg concentrations of adults and juveniles were split into two groups and there was no significant difference by sex (p>0.05). There was a significant difference in fur Hg concentrations of both males and females analyzed by age, respectively (t=-7.373, df=84.33, p=0.000; t=-8.407,

df=64.18, p=0.000), with adult females having significantly higher fur Hg concentrations than juvenile females and adult males having significantly higher fur Hg concentrations than juvenile males (Table 2).

Bats at the GDSNWR exhibited a significant difference in blood Hg concentration by age (t=-3.93, df=148.92, p=0.000), with adults having significantly higher blood Hg concentrations than juveniles (Table 3). There was no significant difference in blood Hg concentrations between male and female bats (p>0.05). When blood Hg concentration of adults and juveniles were split into two groups there was no significant difference by sex (p>0.05; Table 4). There was a significant difference in blood Hg concentrations of both males and females analyzed by age, respectively (t=-2.47, df=76.63, p=0.008; t=-2.85, df=70.90, p=0.003), with adult females having significantly higher blood Hg concentrations than juvenile females and adult males having significantly higher blood Hg concentrations than juvenile males (Table 4).

Fur Hg concentrations of red bats were significantly lower than fur Hg concentrations from the tri-colored bat, evening bat, northern long-eared bat (*Myotis septentrionalis*), southeastern myotis (*Myotis austroriparius*), and big brown bat (F=4.81, df=6, p=0.000; Figure 2). The fur Hg concentration of Rafinesque's big eared bat did not have a significant difference from any other species caught (p>0.05; Figure 2). It should be noted that Rafinesque's big eared bats had a small sample size (n=4).

Blood Hg concentrations of red bats were significantly lower than blood Hg concentrations from evening and tri-colored bats (F=3.03, df=6, p=0.008; Figure 3). Blood Hg concentrations were not significantly different between all other species caught (p>0.05). There was no significant difference in blood or fur Hg concentrations between sample locations at the GDSNWR (p>0.05).

Discussion

Because Hg can bioaccumlate in tissues over time, older bats would be expected to have significantly higher Hg concentrations than younger bats. The results from the current study support this expectation with overall fur and blood Hg concentrations in adults being higher than juveniles. This difference between juveniles and adults is consistent with what has been found in other studies assessing Hg bioaccumulation in bats (Yates et al. 2008, Yates et al. 2009, Divoll et al. 2009).

In a point source contamination study conducted on the South River in Virginia, Hg concentrations have been found to vary significantly in as little as 2 miles (Yates et al. 2007). The fact that there is no significant difference between sample locations at the GDSNWR suggests that the availability of Hg is relatively constant throughout the GDSNWR and suggests that the source of Hg is from atmospheric deposition as opposed to a point source.

Research on Hg exposure in bats is very limited (Reidinger 1972; Petit and Altenbach 1973; Powell 1983; O'Shea et al. 2001). Grippo and Massa, (2000) assessed Hg concentration in bats caught near rivers and lakes with fish consumption advisories in Arkansas. They found fur Hg concentration ranging from 1 to 30 ppm (fw). It is widely accepted that in aquatic ecosystems the bioavailability of Hg increases as pH and dissolved oxygen decrease (Grieb et al. 1990; Suns and Hitchin 1990; Winfrey and Rudd 1990). The bats studied by Grippo and Massa (2000) were located on or near rivers with fish Hg advisories. This would suggest that bats are feeding over more turbid waters with a higher pH and dissolved oxygen level than bats at the GDSNWR. Therefore, bats from the GDSNWR would be expected to have higher Hg concentrations because of the lower pH and dissolved oxygen. Of the 188 fur samples collected at the GDSNWR, only 4.3 percent (8 samples) had Hg fur concentrations above 30 ppm (fw). Nam et al. (2009) assessed Hg concentrations in adult female little brown bats (Myotis lucifugus) from a point source on the South River, Virginia. They found that fur Hg concentrations above 10 ppm (fw) showed a change in brain chemistry. The changes were in hermetic biphasic responses for monoamine oxidase (MAO) and cholinesterase (ChE) activities and muscarinic acetylcholine (mACh) receptor associated with the brains total Hg levels. When applying the lowest observed effect level (LOEL) that Nam et al. (2010) found on the South River to the data set from the GDSNWR 34 percent of bats exceeded the LOEL, suggesting that some bats at the GDSNWR may be experiencing similar changes in brain chemistry.

Due to the lack of sufficient data in lowest observed adverse effect level (LOAEL) for bats, LOAEL concentrations found in white-footed mice (*Peromyscus maniculatus*) by Burton et al. (1977) were applied. Burton et al. (1977) assessed behavioral responses in four populations of mice feeding near the Great Salt Lake, a known Hg hotspot. Swimming ability of mice was ranked on their ability to stay above water, ability to use external body parts and fatigue time. Behavioral responses were assessed by placing mice in a circular floor with grids, activating a loud, constant buzzer and illuminating the field with a bright light. Mice were then ranked based on the amount

of time it took them to leave the center circle, number of times a mouse mad a definite backward movement and number of lines crossed. They found that the sample locations where mice had mean fur Hg concentrations of 7.8 ppm (+/-1.5) (fw) and 10.8 ppm (+/-2.0) (fw) had significant reductions in their swimming and behavior response score compared to sample locations where mice had mean fur Hg concentrations of 0.31 ppm (+/-0.1) (fw) and 1.7 ppm (+/-0.62) (fw). This suggests that the actual effect level is somewhere below 7.8 ppm (+/-1.5) (fw) for white-footed mice. 36 percent of fur Hg concentrations found at the GDSNWR are found to be above the effect level of 7.8 ppm (fw) and 28 percent were above 10.8 ppm (fw). The fact that Burton et al. (1977) found a direct correlation between fur Hg concentration of white-footed mice starting at 1.7 ppm (+/-0.62) (fw) and behavior suggests that the actual impacts of Hg at the GDSNWR are more significant than a LOAEL of 7.8 ppm (+/-1.5) (fw) suggests.

Implications

While bats at the GDSNWR have relatively low mean blood and fur Hg concentrations compared to point source contamination sites, the mean blood and fur Hg concentrations were high compared to reference sites for the same studies. (Nam et al. 2010; Yates et al. 2008; Divoll et al. 2009; Yates et al. 2006). The mean fur Hg concentration at the GDSNWR exceeded LOAEL found by Burton et al. (1977) and when excluding juveniles, concentration in adult bats were above the LOEL found by Nam et al. (2010). This suggests that some bats at the GDSNWR have Hg concentrations that are of concern.

Acknowledgements

We would like to thank Sofia Angelo, Amanda Bessler, Tim Divoll, Dominick Fuski, Casey Huck, John McCloskey, Dustin Meattey, Brad O'Hanlon, Natalie Karouna and Rick Reynolds.

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Figure Legend

Fig. 1 Sample locations at the GDSNWR

Fig. 2 Mean fur Hg concentrations (ppm, fw) from species caught at the GDSNWR. A, AB and B represent significant differences in fur Hg concentrations between species. Box represents 25th

and 75th percentiles with median (solid) and mean (dashed) lines shown. Error bars represent 10th and 90th percentiles

Fig. 3 Mean blood Hg concentrations (ppm, ww) from species caught at the GDSNWR. A, AB and B represent significant differences in blood Hg concentrations between species. Box represents 25th and 75th percentiles with median (solid) and mean (dashed) lines shown. Error bars represent 10th and 90th percentiles

Table 1. Mean fur Hg (fw) concentrations (ppm) in bats caught at the GDSNWR by age and sex.

	n	Mean	Min	Max	SD
Adult	94	13.69	1.9	49.2	9.71
Juvenile	94	4.38	1.09	29.00	3.20
Female	101	6.79	1.09	32.00	5.34
Male	87	11.64	1.1	49.2	10.70
Total	188	9.04	1.09	49.2	8.59

Table 2. Mean fur Hg (fw) concentrations (ppm) of age by sex in bats caught at the GDSNWR.

	Adult				Juvenile					
Sex	n	Mean	Min	Max	SD	n	Mean	Min	Max	SD
Female	45	10.55	2.41	32.00	6.02	56	3.77	1.09	6.7	1.40
Male	49	16.58	1.9	49.20	11.48	38	5.28	1.1	29	4.63

Table 3. Mean blood Hg (ww) concentrations (ppm) in bats caught at the GDSNWR by age and sex.

	n	Mean	Min	Max	SD
Adult	90	0.095	0.025	0.300	0.072
Juvenile	87	0.059	0.016	0.200	0.043
Female	98	0.068	0.017	0.200	0.048
Male	79	0.089	0.016	0.300	0.074
Total	177	0.077	0.016	0.300	0.062

Table 4. Mean blood Hg (ww) concentrations (ppm) of age by sex in bats caught at the GDSNWR.

	Adult				Juvenile					
Sex	n	Mean	Min	Max	SD	n	Mean	Min	Max	SD
Female	43	0.084	0.025	0.200	0.056	55	0.055	0.017	0.135	0.037
Male	47	0.105	0.025	0.300	0.083	32	0.067	0.016	0.200	0.051

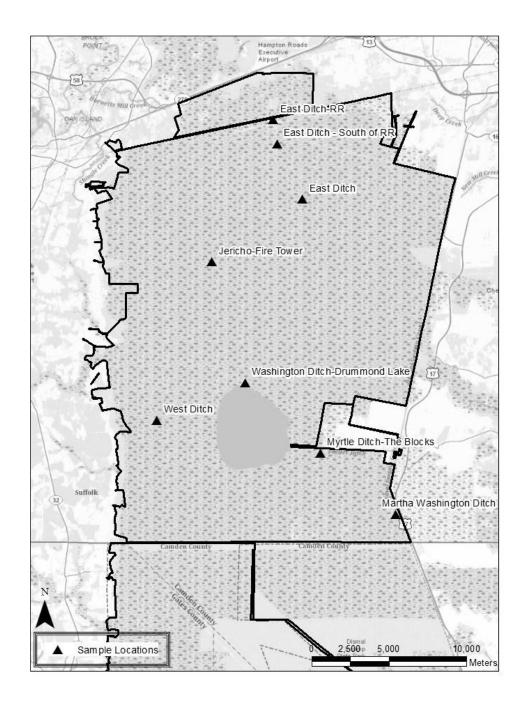


Fig. 1

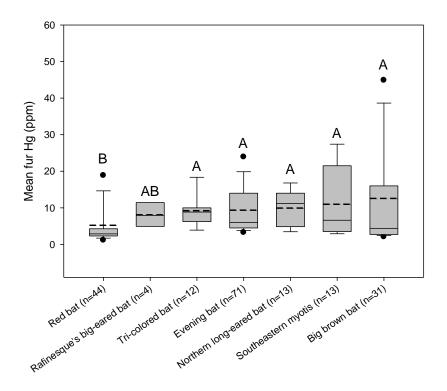


Fig. 2

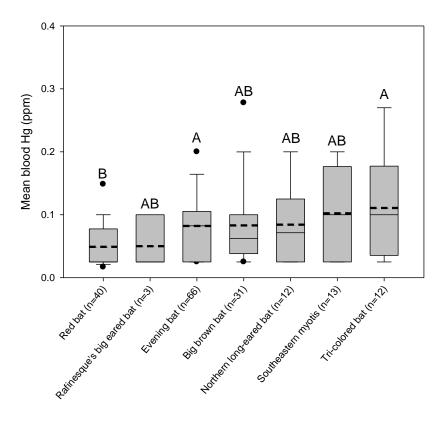


Fig. 3